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Performance of Two Rapid Antigen Detection Tests for Detecting COVID-19 Compared to RT-PCR in Indonesia

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Antigen tests to screen coronavirus disease 2019 (COVID-19) is effective in symptomatic patients, leading to its wide usage in informing whether the person is COVID-19 positive or negative. Our current work had an objective to investigate the diagnostic performance of two antigen-detecting rapid diagnostic tests (Ag-RDTs) which are commonly used in Indonesia. A cross-sectional study was carried out to compare specificity, sensitivity, as well as expected predictive values of Anhui Ag-RDT and Lungene Ag-RDT by comparing the results with that obtained from real-time reverse transcription-polymerase chain reaction (RT-PCR) assay. A total of 98samples were tested for both Ag-RDTs and RT-PCR. The median value of the patients age obtained to be 41.78 years old (interquartile range: 1 to 91 years old). The proportion between female and males was: 52.53% vs 47.47%. The sensitivities of Anhui Ag-RDT and Lungene Ag-RDT were 55.56% and 51.58%, where both Ag-RDTs had specificity of 100%. In conclusion, sensitivity values of Lungene Ag-RDT and Anhui Ag-RDT are similar, where both possess 100% specificity with zero false-positive results. Both of the investigated Ag-RDTs are useful since positive results are likely to be COVID-19 positive.

Keywords: COVID-19; Ct value; Diagnostic Performance; Rapid Antigen Test; RT-PCR.

As of November 2021, coronavirus disease 2019 (COVID-19)has impacted hundreds of millions of people around the world, and is responsible for more than 5.2 million deaths since its first discovery in December 2019 [1].Many countries in the world have been reported the second wave of this severe acute respiratory cases and imposed the national lockdown policy. Of which, Indonesia is also encountering the threat of COVID-19cases. More than 4.2 millions of Indonesian were infected by severe acute respiratory syndrome corona virus 2 (SARS-CoV-2), resulting more than 143,000 deaths as of November 2021[2]. Many efforts have been employed to reduce the number of COVID-19, such as enforcing health protocol law and distributing the COVID-19 vaccine nationally[2]. Unfortunately, there is still no specific anti SARS-CoV-2 drugs have been developed and mutatedSARS-CoV-2 keeps spreading. In this case, prompt diagnosis

and identification of COVID-19 patients who are vulnerable for developing severe courses are important.

The officially recognized diagnosing method for COVID-19 is nucleic acid analysis with real-time reverse transcription-polymerase chain reaction (RT-PCR) as a direct detection method. Although RT-PCR owes a high diagnostic performance for both symptomatic and asymptomatic cases, based on a public health perspective, RT-PCR has some draw backs such as no optimal turnaround time[3], high operational cost, and requiring skilled operator. To answer these challenges, antigen-detecting rapid diagnostic test (Ag-RDT) is employed, where it does not require laboratory capacities and skilled personnel. Moreover, Ag-RDT has a logistic advantage since the diagnostic test could be performed *in-situ*. However, there is a need to investigate the diagnostic performance of Ag-RDT. Hence, in this study, we have compared the results obtained from Ag-RDT with that of the gold standard test (RT-PCR) to determine its sensitivity and specificity.

METHODS

Study designs and ethical approval

A cross-sectional prospective study was conducted at the regional reference COVID-19 diagnostic laboratory, located at Universitas of Warmadewa, Denpasar – Indonesia. Specimens for the Ag-RDT and RT-PCR analysis were acquired by means of nasopharyngeal swab, between June and July 2021. Flocked probe was utilized for the nasopharyngeal swab to collect the specimen, and subsequently dissolved in the universal transport medium (UTM, Copan Diagnostics Inc, US). Specimens were tested before 2 hours had elapsed from their receival in the laboratory. The ethical clearance had been granted by the Faculty of Medicine and Health Sciences, Universitas of Warmadewa (052/Unwar/FKIK/EC-KEPK/VI/2021).

Real-time reverse transcription quantitative polymerase chain reaction

To screen the presence of SARS-CoV-2, the specimen was analyzed using the extraction-free RT-PCR on AccuPrep® Viral RNA kit extraction (Bioneer Inc., Oakland), and nBioCoV-19 RT-PCR kit (Biofarma Inc., Indonesia), where the protocol follows that of the manufacturer's. The specimens were diagnosed for the viral presence through a single-step multiplex RT-PCR on Bio-Rad CFX96TM thermal cycler (CA, US). Amplifications of the viral gene were then observed based on FHEX (internal control), Cal Red 610 (RNA-dependent RNA-polymerase gene), and AM (E gene) fluorophores. Samples showing cycle threshold (Ct) values < 40 were assigned asCOVID-19 positive.

Antigen-detecting rapid diagnostic tests

Two commercial Ag-RDTs included in this study: Anhui (Anhui Deep Blue Medical Technology Co., Ltd, China), and Lungene (Hangzhou Clongene Biotech Co, Ltd, China). Diagnosis using Ag-RDTs followed the instruction provided by the manufacturer.

Data analysis

Results from the Ag-RDTs were compared with that of RT-PCR, which was assignedas a reference standard by the Foundation for Innovative New Diagnostics [4].Sensitivity along with specificity of both investigated Ag-RDTs were taken as a parameter for their diagnostic performance. Youden's J statistic was employed to calculate the cut-off of the Ct value with highest sensitivity and specificity. By employing the variation of hypothetical positivity ranged between 0.5 and 20%, expected negative (NPV) along with positive (PPV) predictive values were quantified, following suggestions from previous reports[4-6].

RESULTS

Demographic data of the subjects (n=98) along with their test results using RT-PCR and two commercially Ag-RDTs (Anhui and Lungene) have been presented (Table 1). This study was participated by male (47.47%) and female (52.53%) subjects with median age of 41.78 years old. As many as 27.27% of the samples were positive by RT-PCR, 84.85 – by Lungene Ag-RDT, and 85.86% – by Anhui Ag-RDT. The mean Ct value of the positive sample was 33.4.

Sensitivity and specificity of Lungene Ag-RDT and Anhui Ag-RDT have been presented in Table 2. The percentage of false-negative results reached 12% in Lungene Ag-RDT, whilst false-positive results were not found. The collective specificity as well as sensitivity of Lungene Ag-

RDT were therefore 55.6% and 100%, respectively. Meanwhile, 51.8% sensitivity and 100% specificity were obtained by Anhui Ag-RDT. The accuracy percentages for Lungene and Anhui Ag-RDTs were 87.9 and 86.9, respectively. The expected NPVs were obtained to be were 99.9%, 99.8%, 98.9%, 97.7% and 94.9% with a variation of positivity rates (0.5%, 1%, 5%, 10% and 20%, respectively). Meanwhile, PPV remained constant 100% at all positivity rates. The optimum value of the Ct cut-off for both investigated Ag-RDTs was 29.

DISCUSSION

The present study compared the sensitivity and specificity between two RDT-Ag tests to screen the availability of the COVID-19 causing virus on the suspected specimen. Lungene Ag-RDT had a higher sensitivity than Anhui Ag-RDT, with 55.6% and 51.8%, respectively. However, the specificity of the two assays was identical (100%) with zero false-positive. These assays are sub-optimally because the ideal test should has a sensitivity of >95% and a specificity of 100% [7]. WHO has set a minimal sensitivity requirement of 80% and a specificity of 97% as acceptable tests[8]. A comprehensive review has documented variations in the estimated sensitivity as well as specificity of the diagnostic accuracy of Ag-RDT assays compared toRT-qPCR[5]. The sensitivity and specificity vary depending on the brand in which the sensitivity and the and specificity of the tests in asymptomatic individualsrangedbetween29-85% and 14-100%, respectively[5].

Previous studies found that the diagnostic tests using Ag-RDT have high sensitivity in those with higher viral loads[5, 9]. For example, the Ag-RDT sensitivity compared to NAAT was higher in symptomatic patients than in those who are asymptomatic (66.7% vs. 47.6 %)[9]. A review found that the average sensitivity of the 48included studies was 72.0% (95%CI: 63.7%, 79.0%). The mean sensitivity decreased over time following the symptoms onset and was lower in the second week (51.0%; 95%CI: 40.8%, 61.0%) than in the first (78.3%;95%CI:71.0%, 84.1%). Better sensitivity was obtained in individuals with a higher load of the virus (Ctvalued"25)compared to those with a lower viral load (94.5% vs40.7%)[5].

In our study, the sensitivity of Lungene Ag-RDT was 55.6%, in which 12 of the 84 individuals who were recorded as negative by the assay turned out to be positive after tested by RT-PCR. Similarly, 13 of the 85 negative tested by Anhui Ag-RDT samples were positive after tested by RT-PCR, indicating that 12.3% of the samples were false-negative. The sensitivity of these Ag-RDT tests might be influenced by the Ct value of the samples. According to Bruzzonea, the question of which RT-PCR cut-off should be employed for deciding whether a sample infected with SARS-CoV-2 or not is still being debated[10]. A study suggested that high viral load was obtained when the Ct value was e"24, while Ct values above 24 indicated a significantly reduced infectiousness[11]. Meanwhile, according to another study summarized in Jefferson's review, a more conservative limit for acceptable RT-qPCR results is in the Ct value range

Table 1. Demographic data of the subject (n=98)

Variable	Number	Frequency (%)
Age		
Min – Max	1 - 91	
Median (SD)	41.78 (19.12)	
Gender		
Male	47	47.47
Female	52	52.53
Antigen Lungene		
Negative	84	84.85
Positive	15	15.15
Antigen Anhui		
Negative	85	85.86
Positive	14	14.14
RT-PCR		
Negative	72	72.73
Positive	27	27.27

Table 2. Diagnostic performance of Ag-RDTs as compared with RT-PCR (n=98)

Diagnostic test	Lungene Ag-RDT	Anhui Ag-RDT
Sensitivity (%)	55.6	51.8
Specificity (%)	100	100
True negative (%)	72	72
False negative (%)	12	13
True positive (%)	15	14
False positive (%)	0	0
Accuracy (%)	87.9	86.9

of 24 to 35[12]. In our present research, the optimal Ct value was observed at the Ct cut-off of 29 with mean Ct value of 33.4 obtained from the positive samples. There are some reasons for the false negative in our samples: (a) some samples might have high Ct values making the viral load was low; or (b) the antigen of the viruses in the samples might had low affinity with antibody used in the tested kit due to mutations of the viruses. To be an accurate testing, the false negative should be low to ensure to be able to detect or diagnose as many as the disease in the population[13, 14].

Our results suggested that despite low sensitivity of Lungene Ag-RDT and Anhui Ag-RDT, these assays still can be used even they do not meet WHO criteria. This is because a positive rapid test sample is most likely to be identified as true positive without further confirmation by RT-PCR and this could help to ease the pressure on health facilities, in particular those with limited resources. As for the negative test, we recommend that RT-PCR need to be tested if the patients showing presumptive for COVID-19. This is in line with the European Center for Disease Prevention and Control's recommendations[6]. Rapid antigen testing is essential in everyday life because it is simple to use, does not necessitate special skills, and has high specificity.

CONCLUSION

Lungene Ag-RDT and Anhui Ag-RDT have similar sensitivity, 55.6% and 51.8%, respectively; however, there was no difference in specificity (100%) with zero false-positive results. Although less than optimal, antigen diagnostics using Lungene Ag-RDT and Anhui Ag-RDT are still important since positive results are mostly positive for COVID-19 and this could help for rapid management of the patients in particular in point-of-care facilities with limited resources.

Conflict of interest

The authors declare no conflict of interest.

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Author contributions

All authors contributed equally to the study, including in data collection, statistical analysis, and data synthesis.

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